Progress Report – FY 2006

Core Name: Pathogen Source Tracking

Project Title: Molecular Techniques for Pathogen Detection

Reporting Period: October 1, 2005 to September 30, 2006

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Background and Rationale:

Coastal and inland waters used for recreation activities and shellfish production are frequently contaminated with human feces and animal manure that carry protozoan parasites and bacteria. Sources of contamination include runoff from agricultural and urban surfaces, effluents from the malfunction of water and waste water treatment plants, storm water drainage, wastewater discharges, and manure from domestic and wild animals occupying buffer areas surrounding rivers and streams. Human pathogens of significant public health concern, such as *Cryptosporidium*, *Toxoplasma*, *Giardia*, and Microsporidia, have been reported in estuarine and marine waters, shellfish, and marine mammals. For this reason, NOAA, USDA, and CDC are collaborating to investigate the presence and abundance of these pathogens in the coastal environment.

Traditionally, microscope and antibody based techniques have been used to identify the aforementioned protist pathogens. However, neither of these methods can identify the species and/or genotypes that specifically infect humans or provide necessary sensitivity particularly at low levels of parasite abundance. Such identification now requires the use of molecular tools such as polymerase chain reaction (PCR) and gene sequencing. Using established methodology, USDA and CDC laboratories will design and test newly developed pathogen primers for specificity. Molecular-based assays using real-time polymerase chain reaction will be evaluated against existing microscopy and conventional PCR techniques for the detection and prevalence of the pathogens in coastal ecosystems. The combination of PCR, gene sequence analysis, and bacterial source tracking techniques will allow investigators to assess levels of pathogen contamination from environmental samples and allow a comparison of the identified pathogen sources by molecular techniques with those of fecal bacteria from the same sites to better understand routes of fecal contamination and accumulation in coastal areas.

Objectives:

- Develop new diagnostic techniques to detect and to the extent possible determine the potential sources of the primary *Cryptosporidium*, *Giardia*, *Toxoplasma*, and Microsporidia species or genotypes that infect humans and determine best methodology for pathogen detection by laboratory testing of new and old

techniques. Gene sequencing will be used to narrow the list of potential sources of protozoan pathogens; bacterial sources will be identified using library based methods.

- Conduct a regional field study at historical *Cryptosporidium* sampling stations in the Chesapeake Bay watershed to test the newly developed techniques on environmental samples and to develop a regional baseline of prevalence and source of contamination
- Conduct a nationwide field study of coastal waters, shellfish, sediment, and marine mammals to determine the prevalence, pathogen burden, and possible sources of selected environmental pathogens
- Conduct workshops to transfer technology to investigators at the NOAA Charleston Center of Excellence, other scientists, shellfish managers, environmental managers, shellfish growers, and public health agencies

Accomplishments: (This project received no OHHI funding for FY 2006)

- Completed design and preliminary laboratory testing of PCR primers for *Cryptosporidium, Giardia*, and *Toxoplasma*. New PCR techniques detected 90 and 100% of samples spiked with 100 and 1000 *Cryptosporidium* oocysts respectively compared to 20 and 74% detection by the traditional Immnofluorescent Antibody Assay (IFA) method.
- Optimized recovery, DNA extraction and PCR techniques for pathogen detection in laboratory studies using spiked samples in water and sediment collected from Chesapeake Bay. Current PCR methods being tested are not quantitative, but based on a presence or absence of the pathogen in a sample. However, positive findings are believed to be infectious for humans based on successful detection of 5 to 100 oocysts (*Cryptosporidium*) and cysts (*Giardia*) in spiked samples. A single oocyst is believed capable of causing infections in some individuals and Okhuysen et. al (1999) reported a dose of approximately 87 oocysts as capable of causing infections in 50% of individuals exposed to *Cryptosporidium*.
- Initiated building DNA libraries for antibiotic resistance and bacterial source tracking protocols. Library currently includes resistance information for bacteria from four sewage treatment plants and approximately 20 wild and domesticated animals.
- Began field tests for detection of selected pathogens and fecal bacteria in environmental samples taken before and after tertiary treatment at sewage treatment plants and in sediments along transects up and downriver of outfalls of sewage treatment plants rated at low, moderate, and high levels of effluent discharge. Twenty stations sampled in three different river systems during May and June were evaluated for pathogen detection using conventional PCR and fecal bacterial source using antibiotic resistance analysis. *Giardia duodenalis assemblage A is infectious for humans and other primates as well as cattle, sheep, deer, horses, pigs, and ferrets. Giardia assemblage A was detected in the effluent of 5 of 7 samples taken at treatment plants with* moderate and high volume discharge. *Cryptosporidium andersoni (*primary source adult cattle) was detected in 3 of 4 samples from high volume discharge facilities. No *Giardia* or *Cryptosporidium* was detected from effluent at the low volume discharge site.

- Conducted first quarterly cruise to detect pathogens and antibiotic resistant bacteria in water, sediment, and shellfish at 13 locations around the Maryland portion of Chesapeake Bay. Samples were evaluated for pathogens using conventional PCR and fecal bacterial source using antibiotic resistance analysis. *Giardia* sp. (assemblage A) was found in water and sediment at one of 13 sites and in the digestive diverticula from a pool of 5 oysters at a second site. Both sites are in proximity to sewage treatment plants. *Cryptosporidium* was not detected at any of the 13 locations during the summer quarterly sampling effort.

Planned Activities for 2007 (Pending Available Funding):

- Present status of the NOAA OHH program and results of the current study to the Interstate Shellfish Seminar and Northeast Shellfish Sanitation Association in Cape May, NJ October 2006.
- Present project update to an OHH meeting of principal investigators in Charleston, SC (TBD)
- Continue quarterly regional field studies for detection of selected pathogens in water, sediment, and shellfish
- Sample marine mammals as opportunities arise
- Attend annual OHH meeting of principal investigators
- Respond to special sampling opportunities immediately after large rainfall events (such as tropical storms) in the Maryland portion of Chesapeake Bay as chances arise

Publications/Presentations:

A poster "Molecular Techniques to Detect Emerging Human Pathogens In Coastal Ecosystems" was presented at the HML Center of Excellence principal investigators meeting, Charleston, S.C. January 2006

A presentation "NOAA's Oceans and Human Health Initiative - An overview of the Microbiology Program" was presented at the Interstate Shellfish Seminar, Ocean City, MD. October 2005

Application/Technology Transfer relevant to OHH Strategic Goals:

1.0 Scientific Research and Application

Cryptosporidium, *Giardia*, and *Toxoplasma* were selected for study because they: 1) originate from human and animal feces, 2) have demonstrated health affects on mammals (including humans), 3) have been reported in shellfish and/or marine mammals, 4) do not replicate outside of the host and therefore may serve as indicators of recent fecal contamination, and 5) may serve as a potential source of human exposure from the consumption of raw shellfish harboring these pathogens or by the use of waterways for recreational activities. Development of these tools will allow scientists, industry and environmental managers to assess the burden of these organisms in coastal environments and provide a baseline for developing management plans. The fate of the organisms, the best medium to sample for detection of the selected pathogens, and links to other fecal indicator organisms

such as bacteria in the coastal and estuarine environment are unknown. Successful completion of this project greatly increases the scientific understanding on the impact of the selected organisms on coastal ecosystems and allows more complete future investigations into the health of coastal ecosystems. Currently there is no tool that can be used to quickly and accurately detect and quantify the abundance of the selected organisms in water, sediments and shellfish under field conditions. This project attempts to provide new techniques to improve the detection and ultimately allow quantification of these organisms.

2.0 Public Information and Outreach

This and similar projects that have a public health link to shellfish are of strong interest to the Interstate Shellfish Sanitation Conference (ISSC), shellfish managers, growers, local public health groups, and consumers. This project helps develop techniques that can be employed to assess the risk in consumption of raw shellfish from areas that may harbor or accumulate human pathogens. In the recent years the ISSC has been made aware of this work and has provided logistical support and contacts used in making collections for evaluation. This association is important to maintain toward the end goal of conducting a national baseline survey of protozoan pathogens and fecal bacteria in shellfish and growing areas. Project investigators continue to provide communication to annual meetings of the Interstate Shellfish Seminar and Northeast Shellfish Sanitation Association. Upon project completion technology transfer to users is planned through convening an ISSC workshop.

3.0 Capacity Building

With a strong USDA and CDC partnership, this project has developed new capability to detect targeted human and environmental pathogens from water, sediment, and shellfish that with further testing can be transferred to and implemented by NOAA and associated research laboratories after field validation.

Project abstract:

Traditionally, microscope and antibody based techniques have been used to identify pathogens such as those selected for study in this project. However, neither of these methods can identify species that specifically infect humans. Such identification requires the use of molecular tools such as polymerase chain reaction (PCR) and gene sequencing. In addition, bacterial source tracking technology, such as antibiotic resistance analysis, is a basic tool in identifying human and nonhuman fecal sources from environmental samples. The project objectives which seek to transfer source tracking technology from CCEHBR Charleston to CCEHBR Oxford, develop new diagnostic techniques for the detection of the primary *Cryptosporidium*, *Giardia*, *Toxoplasma*, and Microsporidia species or genotypes that infect humans have essentially been accomplished. What remains is to validate recovery of these organisms in environmental samples, build a baseline of these pathogens in sediments, water, and shellfish in Chesapeake Bay and nationally, to ultimately develop an accurate quantitative methodology for pathogen detection, and transfer technology to targeted users.

Unresolved Issues:

- Current budget process causes strong challenges in project continuity. Funding commitment needs to be multi-year, occur early in the spending cycle to allow available funds throughout the year to support field and partner research, and to allow the purchase and use of time sensitive reagents throughout the year. Completion of this project will not be possible without restoration of funding.
- Bacterial pathogens may be found at high levels in some coastal areas and often thousands of organisms must be present to initiate human infections. By contrast, the targeted protozoa only need 1-100 organisms to infect humans and therefore, to optimize detection, they must be concentrated from environmental samples that often contain few infective particles per similar sample size
- Improvements in the field of quantitative PCR technology to allow meaningful numeric reporting of pathogens of low abundance in a sample. Quantitative PCR is based on an estimation of the number of organisms present based on the amount of pathogen DNA replicated from a sample. Currently it is not viewed as meaningful to report results of pathogen abundance in a numerical format for pathogens that cause disease by ingestion of so few infective particles (1-100 oocysts).

Budget Report:

This Project Was Scheduled to Receive $60.1K in OHH Funding in FY2006, But Received No FY06 OHH Program Support. Project accomplishments were made by very limited support in base funding from the NOAA NOS budget for the CCEHBR – Oxford Laboratory, USDA Beltsville Laboratory, and CDC.

Budget Report - Use of Molecular Techniques to Detect Emerging Pathogens in Coastal Ecosystems
Pathogen Source Tracking, Infectious Disease
NOAA Oceans and Human Health Initiative 2004-2006

	OHH Project Budget (Reported in $K)			
ITEM	**FY 2004**	**FY 2005**	**FY 2006**	**Total OHH Funding**
Supplies				
Source Tracking Supplies	5.0	4.3	0.0	9.3
USDA Molecular Supplies	10.0	12.0	0.0	22.0
CDC Molecular Supplies	10.0	10.0	0.0	20.0
Marine Mammals	1.0	0	0.0	1.0
Section Total	26.0	26.3	0.0	52.3

Travel	0	2.0	0.0	2.0
Equipment	24.0	12.7	0.0	36.7
Summer Student	0	10.6	0.0	10.6
Sample Collection	0	7	0.0	7.0
Other Indirect Costs	0	1.5	0.0	1.5
Total Expenditures	50.0	60.1	0.0	110.1